REMARKS

Claims 9-18 are pending and under Rejection in the application. In the parent application, claims 9-18 were previously cancelled without prejudice due to restriction, and the claims prosecuted (1-8) were ultimately allowed to issue as U.S. Patent No. 6,656,685. Original claims 9-18 were reintroduced in this divisional filing. In this Response, claim 9, the sole independent claim, is amended to incorporate the limitations that issued in claim 1 of the '685 patent. Claims 15-17 are cancelled herein.

I. Rejection of claims 9-18 under 37 CFR Section 112/2d Paragraph

The Examiner rejected claims 9-18 on two basis, the first for being indefinite because the preamble recites an automatic method, yet the method steps allegedly do not involve automation. Applicants have amended claim 9 to add "automatically" prior to the single step remaining after amendment. Applicants request reconsideration of the application on that basis.

The Examiner has also rejected claim 16 for being indefinite. Claim 16 has been cancelled herein, and its subject matter has not been reintroduced elsewhere. Therefore, the rejection is moot.

II. Rejection of claims 1-2, 4-5 under 35 USC Section 102

The Examiner rejected claims 9-15 under 35 USC 102(b) as being anticipated by Schwartz US 4,886,741.

Schwartz et al. is disclosed and discussed by applicants on page 4 of the application. US Patent No. 4,886,741 (Schwartz et al.) describe the use of dextran sulfate, sodium salt, for use as a volume exclusion agent for ISH. The average molecular weight is not described, but by reference to the source (Sigma Chemical, Products for Life Sciences, St. Louis, MO) it has an average molecular weight of 500,000. Schwartz et al. also disclose that dextran sulfate is typically used at a concentration of about 5-10% (w/v). Contrary to the Examiner's assertions, Schwartz et al. do **not** disclose *low molecular weight* dextran sulfate for use as an exclusion agent in polynucleotide hybridization. In fact, Schwartz et al. describe three different types of volume exclusion agents, including polyethylene glycol, anionic polymers of polyacrylate or

polymethylacrylate, and generic dextran sulfate. It is not clear from the reference what molecular weight ranges cited apply to which categories of volume exclusion agents, but it is clear that low molecular weight dextran sulfate is not specifically called out either in col 3. lines 1-23, or any of the other places cited by the Examiner. The reference repeatedly mentions "10% dextran sulfate" (col. 9, line 26; col. 10, line 24-25) but does not specify the molecular weight. In the absence of any indication to the contrary, one of ordinary skill would assume that the dextran sulfate being used was the 500,000-2,000,000 MW version, which is what is normally available in labs.

Applicants have limited the sole independent claim, claim 9, by adding the low molecular weight range specified, and therefore distinguish over Schwartz et al. Therefore, the claims as amended are not anticipated by the reference. Applicants also point out that the claims are now limited to "**automated** in situ hybridization" which further distinguishes the claims over Schwartz et al. which teaches a manual procedure.

Applicants have more clearly defined their invention by changing the form of the claim to Jepson format, thereby defining the background of the invention and placing the improvement in that perspective. The preamble defines the automated environment in which the claimed hybridization buffer finds it utility, and therefore distinguishes over both Schwartz et al. which recites manual hybridization procedures. The Ventana-automated tissue staining environment includes the unique (and patented, see US 5,225,325) LIQUID COVERSLIP™ method to inhibit evaporation of liquid off the tissue or target during incubation at the high temperatures required for *in situ* hybridization. In particular, method claim 9 has been amended to add "said method executed in an automated staining system having evaporation inhibitor liquid covering a polynucleotide hybridization buffer-covered target on said slide." This language finds support on page 8, lines 24-31 wherein Applicants discuss the automated mixing environment by use of the LIQUID COVERSLIP™ (essentially a layer of mineral oil covering the aqueous layer over the tissue or taregt) with air jets to rotate/counter-rotate the solutions on the slide, thereby effectively mixing the aqueous phase. It was discovered by Applicants that standard hybridization buffers did not operate well in Ventana's environment due to the high viscosity of these buffers. The high viscosity inhibits the mixing and dispersion of the probe reagents when dispensed through the mineral oil layer into the buffer/tissue layer. Consequently, Applicants defined the problem (high viscosity), and then found the solution-a low-viscosity buffer that would still have the

necessary volume-exclusion effect for optimal hybridization. Not only does the Schwartz reference not teach low-molecular weight buffers, they do not appreciate Applicants' fundamental discovery: that high viscosity inhibits hybridization in Ventana's automated environment.

It is fundamental patent law that in order for a reference to anticipate a claim, each and every element of the claim must be present in the reference, either explicitly or through principles of inherency. Therefore, the claims as amended are not anticipated by Schwartz et al. since the claims now include the specific automated environment. Reconsideration of the application is therefore respectfully requested.

III. Rejection of Claim 16 under 35 USC 103(a)

The Examiner has rejected now-canceled claim 16 as obvious in light of Schwartz et al. US 4886741 and Gray et al. US 5447841. The rejection is moot given the cancellation of the claim.

IV. Rejection of claims 17 and 18 under 35 USC 103(a)

The Examiner has rejected now-canceled claim 17 and still-pending claim 18 as obvious in light of Schwartz et al. US 4886741 and further in view of Towne et al. US 6,855,552, issued 2/15/05. The rejection as to claim 17 is moot given the cancellation of the claim. Applicants traverse the rejection of claim 18 for the following reasons.

Claim 18, which depends directly from claim 9, as now amended recites "The method of claim 9 wherein said probe composition is arrayed on said solid substrate." The only change was to replace "a" with "said."

Since claim 9 has been amended to add the low molecular weight range limitation, the claims require the use of low molecular weight dextran sulfate to effectuate the exclusion effect in the Ventana instrument environment. However, claim 18 introduces the limitation that the probe be located on the substrate surface, ala a nucleic acid microarray. This limitation is supported in the specification, p. 19, in Example 6 and Figure 5, which shows hybridization to a ClonTech Human Atlas DNA microarray, and on pages 10, line 30 through page 11, line 9. Microarrays are another form of solid substrate that may be run on a Ventana system for automatically hybridizing, and claim 18 is intended to address that situation.

Towne et al. is directed to aqueous buffer compositions and methods for antigen retrieval in formalin-fixed, paraffin-embedded tissue, for automated methods for deparaffinizing tissue without the use of organic solvents, and automated methods for simultaneous deparaffinization and antigen retrieval. Towne et al. do disclose that a tissue array may be a biological sample, but not in the sense the Examiner has used it. It is commonly understood by one of ordinary skill that a tissue microarray comprises multiple individual tissue pieces that are organized in an array on a microscope slide, and to perform in situ hybridization on the tissue one would introduce the probe composition in a liquid format onto the tissue and then perform the heating, cooling and washing procedures required to hybridize the probes to the DNA targets within the tissues. Claim 18 is directed to the opposite arrangement whereby a DNA microarray is being used, which contains its DNA probes affixed to the slide surface, and the sample is contacted with the surface-bound probes to effect hybridization. Therefore, Towne et al. does not provide the element of having the probe composition arrayed on a solid substrate.

Applicants respectfully assert that the rejection of claim 18 is deficient, and therefore there is no prima facie case of obviousness. Applicants request reconsideration of the application on that basis.

Respectfully submitted,

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